

STUDIES ON A-AVITAMINOSIS IN CHICKENS

I. LESIONS OF THE RESPIRATORY TRACT AND THEIR RELATION TO SOME INFECTIOUS DISEASES

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PLATES 21 TO 23

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The diseased condition of chickens resulting from a lack of vitamin A in the diet is probably the most important of the nutritional diseases caused by A-avitaminosis in domestic animals. As pointed out by Beach (1) and Seifried and Schaaf (2), it causes serious losses on poultry farms. The clinical and anatomical picture closely resembles that found in some of the most important infectious diseases of fowls, chicken pox, coryza contagiosa, and infectious bronchitis. Golblatt and Benischek (3), Tyson and Smith (4), and Wolbach and Howe (5) have described the histological changes following a lack of vitamin A in the diet in guinea pigs, rats, and in one human individual. The characteristic changes are the substitution of stratified keratinizing epithelium for normal epithelium in various parts of the respiratory tract, alimentary tract, eyes, paraocular glands and the genito-urinary tract. No studies have been made of the histological changes in A-avitaminosis in fowls. It is the purpose of this paper to report such studies and to compare the changes with those found in certain infectious diseases.

Material

The material used consisted of 16 cases of A-avitaminosis, of which 12 were produced experimentally and 4 were spontaneous. The diet employed for the experimental production of the disease was identical with that used by Beach and

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TABLE I
Showing Source and Kind of Material Used

Number of chicken	Food received	Age at beginning of experiment	First symptoms after	Dead after beginning of experiment	Gross lesions											Histological findings																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
					Days	30 days (Killed)	40 days	45 days	48 days	44 days	52 days	51 days	58 days	54 days	77 days		56 days (Killed)	81 days (Died suddenly?)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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* Main lesions in submaxillary and lingual glands.

by us in our earlier experiments. It consisted of 50 parts wheat bran, 50 parts wheat shorts, 50 parts ground barley, 10 parts meat scrap, 10 parts fish scrap, and 20 parts soybean meal. We used chickens about 3 months old, for according to our earlier experiments and in common with other investigators, we have observed that a much shorter period is required to produce the characteristic symptoms followed by death in young, partially grown animals, than in adults. This is brought out in the present experiments in which Chickens 11 and 12 (see Table I) that were about 3 months older than the other animals, developed symptoms much later and to a minor degree than did the younger ones. To insure the presence of vitamin D, all animals were exposed daily to sunlight or to ultraviolet light. The six controls (Nos. 17-24) received the same food mixture and in addition plenty of greens (alfalfa, cabbage) and 3 cc. of cod liver oil daily given with a dropper. All of the controls remained well, failing to show any gross pathological or histological lesions. No symptoms of rickets or of a deficiency of vitamin B were observed at any time during the experiment. Table I gives all details of the experiment together with the gross pathological findings.

Methods

Most of the tissues were fixed in 10 per cent formalin immediately after autopsy, but in a few cases Zenker's fluid was used. The tissues from the nasal passages and the sinuses were decalcified, some in 5 per cent nitric acid and some in 5 per cent formol-nitric acid. All tissues were imbedded in paraffin, and for special purposes frozen sections were made. For routine stains hematoxylin and eosin, van Gieson's stain, and Mallory's methylene blue phloxine, as modified by Davidoff (6) and McGregor (7), were used. In addition the following stains were used for special purposes: methylene blue, Gram's and Giemsa's stain,—the best results being obtained with the last, with tissues fixed in formalin as well as in Zenker's fluid, by the modification given by Wolbach,—Weigert's elastic tissue stain, Weigert's fibrin stain, fat stain with Sudan III and Scharlach R, oxydase reaction, the stains with methylgreen-pyronin and fuchsin-light green, with mucicarmine (Mayer), thionin, toluidin, cresylecht-violette, and Pasini's method with waterblue-orcein, which gave distinct results in staining the keratohyalin globules, and finally stains with ferric sulfur, calcium permanganate, Congo red (keratinization), and Hammerschmidt's stain for Guarnieri's inclusion bodies.

Gross Pathology

Nasal Passages and Sinuses.—Sagittal and gross sections through the nasal cavities and the communicating sinuses show all stages of a disturbance of the lining of the nostrils and cleft palate.

Early in the disease the turbinate bones (concha superior and inferior) are filled with seromucoid, water-clear masses, which by the application of slight pressure

can be forced out of the nostrils and cleft palate. Owing to the consistency of the exudate and the complicated structure of the nasal fossae, the vestibule becomes plugged and the exudate overflows into the paranasal sinuses, often early in the disease. On the other hand, the exudate may be forced through the cleft palate, filling up the region around the vomer with seromucoid masses. Later this exudate becomes transformed into white or slightly yellow caseous masses which are found between the turbinate bones, in the nasolacrimal duct, nasal vestibule, and sinuses, causing more or less swelling of the face (Figs. 1 and 2). This involvement of the nasal passages and the sinuses may be uni- or bilateral. If on one side, the turbinate bones and the sinuses may become so enlarged that they extend over the median line and compress the opposite nasal fossa. The characteristic eye lesions in this disease, which will be described in another paper with M. Westhues, are complicated by the blocking of the nasolacrimal duct. When the sinuses are completely filled, the bulbus of the eye, which cannot move in a ventral direction, is pressed against the os frontale and sometimes forced out in a lateral direction. The protrusion of the palate into the cavity of the mouth, often observed in virus-diphtheria, is not usually seen in A-avitaminosis. After the inflammatory products have been removed, the mucous membranes throughout the nasal passages and sinuses show a peculiar, thin, rough and dry membrane, which may also be found in the mouth and throat. Unattached masses of white caseous material are often present in the cleft palate and in the surrounding mucous membrane of the roof of the mouth.

Larynx and Trachea.—Almost regularly in A-avitaminosis of chickens marked lesions occur also in the larynx, trachea and bronchi. As shown in the table, they are found in the early as well as in the later stages of the disease.

Chickens 11 and 12 on a diet lacking vitamin A showed no gross lesions, but microscopically early changes were found in the glands of the mouth, esophagus and trachea. Those in the trachea were apparently the most marked and were probably the oldest. The lesions in the trachea have as yet received no attention, but we feel that they were of special importance as it is so difficult to distinguish them from those commonly found in roup, diphtheria, and infectious tracheitis. In addition to the lesions near the entrance of the pharynx, consisting of pustule-like patches which are due to collections of white caseous material in the mucous glands, glandula cricoarytenoidea, the pathologico-anatomical picture is characterized by a peculiar laryngitis, tracheitis, and bronchitis. The mucous membrane of the anterior end of the larynx on its ventral side and in the pointed angle which is formed by the cartilages of the larynx, often shows caseous crumbly white masses. Similar lesions are frequently found throughout the entire length of the trachea and often in the bronchi. In very early stages, however, they are much less marked and may be difficult to see. The mucous membrane is then covered with a fine

film or haze; it is dry, dull, and slightly uneven on the surface, whereas the normal membrane is moist. In other cases small nodule-like particles may be visible in or beneath the mucous membrane, especially in the upper part of the trachea. In later stages the gross lesions are much more striking and may easily be seen with the naked eye. More or less thin membranes cover the mucous membrane throughout the entire length of the trachea and the bronchi. In most cases these membranes are pulled off the underlying mucosa, thus forming a thin-walled continuous tube within the trachea and bronchi (Fig. 3), a condition that might be mistaken for infectious tracheitis. The smaller bronchi very often become completely plugged with these membranes causing bronchiectasis. In some cases the larynx shows the most marked changes while in others the trachea is more involved.

Histopathology

Larynx, Trachea, and Bronchi.—The changes in these structures are the more easily understood as the normal anatomy is less complicated than in the nasal fossae and adjoining sinuses.

The first lesion is in the columnar ciliated epithelium and is characterized by an atrophy of the cytoplasm and a loss of the cilia. Along with this protoplasmic change, the nuclei often present more or less marked karyorrhexis. The atrophying and degenerating ciliated cells hang like tufts on the basement membrane and later are pushed off and may form a pseudomembrane.

While this process is going on, there appear as islands beneath the original epithelium new cylindrical or polygonal cells either singly or in layers. These are especially marked in the trachea where they may appear as focal syncytial masses (Fig. 4). The cells evidently have their origin in the original columnar epithelium. They differ from the original cells in that their nuclei stain more deeply with basic dyes, and the presence of numerous mitoses indicates that they are dividing rapidly. Mitotic figures are especially numerous in the deeper layers of this newly formed epithelium. As these new cells become more numerous and form layers beneath or in place of the original epithelium, the cells nearer the surface are more and more flattened, the nuclei become larger and contain less chromatin. Later the superficial cells are quite flat, the cytoplasm is homogeneous and the nuclei are lost (Figs. 5 and 6). In this stage the cell boundaries become less clearly defined and at the surface the cells are desquamated as hard, dry, flattened scales. Pasini's waterblue-orcein method shows keratohyalin granules scattered irregularly in the cytoplasm of these cells. In the outer layers these granules sometimes fuse and form hyalin masses of eleidin. The process resembles very closely the incomplete keratinization of the mucous membranes, especially that of the esophagus and certain parts of the tongue of chickens. The columnar ciliated epithelium lining the trachea and bronchi and the epithelium of the submucous glands thus become transformed into squamous stratified keratinizing epithelium with typical intercellular bridges. Owing to the fact that the keratinized epithelium extends as a

continuous layer into the glands of the submucosa, the picture strikingly resembles that of the mucous membrane of the cavity of the mouth, and to a less extent of the normal skin. This is brought out in Fig. 6. Eosinophilic leucocytes which are normally found in the keratinized epithelium of the tongue and esophagus appear either scattered or in small foci. Other cells which may be associated with secondary bacterial infections also appear.

Along with the formation of this keratinized epithelium the original columnar cells are thrown off either singly or in layers. Some of these cells may appear practically normal, but the majority lose their cilia, the protoplasm takes the routine stains less intensely, and the cells later become distended so that they have the appearance of a ball or a balloon (Fig. 7). The chromatin in the nuclei of these cells becomes irregular and fragmented, and later only a few chromatin knots remain scattered irregularly in the balloon-shaped cytoplasm. Finally the cells appear as more or less homogeneous shadows, the whole process being that described by Unna as "balloon degeneration." This balloon degeneration involves not only the original epithelium but also the squamous keratinizing cells. Occasionally these degenerating cells may fuse and form what may be called "balloon giant cells." The cell boundaries may still be visible in these unusually large giant cells.

In the deeper layers of the new epithelium a reticular degeneration of cells may be found. The cells are enlarged, the cytoplasm is occupied by round vacuoles, sometimes to such an extent that it is only visible as a reticular network, the nuclei show numerous chromatin clumps, and nuclear material may be extruded into the cytoplasm. This type of degeneration is not as common as the balloon type.

The degenerating cells become separated and collect in the lumen of the trachea and bronchi in the form of a membrane in which numerous bacterial colonies appear. These bacteria may invade the cells and in cases become so numerous that the cytoplasm is filled with them and the nuclei sometimes destroyed (Fig. 7).

Stains with Sudan III and Scharlach R of the trachea at various stages of the degeneration show practically no fat. In a few cases a small amount of fat was present but this type of degeneration is far from common.

As the keratinization of the epithelial cells of the glands in the trachea progresses, the presence of mucus, as shown by special stain, becomes progressively less. In the early stages one may find keratinized epithelium in one portion of the gland and secreting mucus cells in another. Later, as the keratinized epithelium replaces the true glandular epithelium, mucin is found, if at all, in very small granules in the center of what remains of the original epithelium. When the glands become completely filled with keratinized squamous epithelium mucin is absent.

Associated with the loss of cilia and the decreased or absent secretion of mucin one would expect an invasion of the mucosa with bacteria. Organisms are found not only on the surface of the epithelium and between the epithelial cells but also in the glands of the tunica propria, in the tunica propria itself, and even in the submucosa. The degenerating cells of the epithelium soon become invaded by

bacteria and leucocytes may make their appearance. As the disease progresses bacteria and signs of inflammation become less marked. However, signs of bacterial infection are not always present, and in the fowl we feel that the keratinization of the epithelium is not related to the bacterial infection. In the rat, Tyson and Smith (5) came to the opposite conclusion.

In nearly all cases, a more or less marked cell infiltration in the tunica propria, partly around the numerous vessels, partly in a more diffuse distribution, is found (Figs. 5 and 6). According to the results of control investigations, the occurrence of lymphoid tissue in the tunica propria of the trachea is a normal finding, but in cases of A-avitaminosis its collection is so massive that there is no doubt of its pathological significance. The greater part of these infiltration cells are lymphocytes, to which are added typical histiocytes and a few plasma cells. In most cases some eosinophile leucocytes, red blood corpuscles and occasionally neutrophile leucocytes have also been found. In some early stages with extreme degeneration and proliferation of the original epithelium and a distinct edematous condition of the underlying propria, the occurrence of peculiar large cells, a type of histogenous mast cells, has been observed, especially in the upper layers. They are elongated or oval with a longish-oval nucleus which is rich in chromatin and sometimes shows a typical wheel form. Using Giemsa's stain, very small blue or blue-violet granules can be found in their cytoplasm. In general one has the impression that the upper layers of the tunica propria, situated directly beneath the epithelium of the surface, and the neighborhood of the glands are occupied more densely with these infiltrating cells than its deeper parts. Sometimes the reverse is true; in fact, the degree of the infiltration varies greatly in different cases. Mitoses are very seldom recognizable in these cells. Moreover, lesions may also be found in the endothelial cells of the infiltrated vessels, the lumina of which are frequently filled with lymphocytes. There is more or less swelling of these cells and a change in the chromatin structure of their nuclei. In some preparations the elastic fibers seem to be thickened and more separated. Usually these lesions are associated with edema. In the remaining parts of the trachea no particular changes could be discovered.

The same complex of lesions described in the trachea is also present in the bronchi and bronchioli. They are sometimes filled with desquamated keratinized epithelium, so that bronchiectasis is the natural sequence.

Nasal Cavities and Communicating Sinuses.—The lesions of the nasal passages and their sinuses have been studied on cross sections cut perpendicular to the roof of the mouth at several points between the nostrils and the posterior limit of the nasal cavity or bulb of the eye. The evolution, course, and final stage of the process are exactly the same as previously described in the trachea (Figs. 8, 9, and 10). It may be pointed out that in general all parts of the nasal cavities are

involved and that only some chronological differences in the involvement of the several regions can be observed. In the gland-free part of the nasal vestibule, *regio vestibularis*, lesions have been determined in all cases, not so much in the form of a real keratinization but rather of an increased proliferation of the superficial epithelial cells.

Typical and distinct lesions, as described in the trachea, are found in the respiratory mucous membrane of the roof of the nasal vestibule and especially in the median and dorsal area of the part known as the concha of the vestibule. One obtains the impression that the entire process in this and other parts of the nasal cavities (olfactory portion, respiratory portion) (Figs. 8, 9, and 10) begins in the mucous membrane epithelium and that the epithelium of the glands becomes involved a little later.

In addition to numerous keratohyalin corpuscles in the cells of the superficial layers of the newly formed squamous stratified epithelium and the appearance of eleidin (Pasini's method), the following lesions in the nuclei of the keratinizing cells have been encountered in the nasal cavities:—As a rule it has been observed, even in the deeper layers, that marked alterations of the nuclei are coexistent with the formation and appearance of keratohyalin granules in the cytoplasm. There is especially a marked fragmentation and extrusion of nuclear material (nucleoli) and furthermore a relative increase in the volume of the cytoplasm as compared with nucleus (Fig. 11). According to the investigations of Ludford (8), the same lesions may take place in the skin under normal conditions, while in a hypertrophied epidermis, under pathological conditions, these nucleolar changes are still more marked. It seems very probable, therefore, that the nuclear substances and particularly the nucleoli are connected in some way with the process of keratinization and the formation of keratohyalin granules. In addition the nucleoli after extrusion from the nuclei not infrequently change their staining properties. When stained by Hammerschmidt's method they resemble Guarnieri's inclusion bodies. Moreover, the nuclei of these cells from which the nucleoli have been extruded have the characteristic shape of nuclei in cells containing Guarnieri's bodies. It is worthy of note that in the pathological keratinization associated with chicken-pox Eberbeck (9) found the same type of lesion.

The sinuses communicating with the nasal cavity show essentially the same lesions as in the epithelium of the nose and trachea. These lesions are modified depending upon the anatomical structure of the cavity itself. In the paranasal sinuses the mucous membrane is thinner and contains only a few glands on the median wall, contrary to the opinion of Bittner (10). Keratinization occurs in the nasolacrimal duct and in the excretory duct of the lateral nasal gland, *glandula lateralis nasi*. The lateral nasal gland itself is only slightly involved,

the main lesion being in the ducts and collecting space while the acini themselves appear normal.

Bacterial invasion of the degenerating epithelium occurs in the nose just as it does in the trachea (Fig. 12). It is, however, more marked and the nasal cavity and fossa may become filled with degenerated cells and purulent exudate. The tunica propria becomes involved and in addition to the infiltrating cells one may find a slight edema and a swelling of the endothelial cells of the blood vessels.

Relation of A-Avitaminosis to Some Infectious Diseases of the Upper Respiratory Tract

The great variability of the clinical symptoms and pathological lesions in A-avitaminosis of the respiratory tract may make it difficult to differentiate this condition from coryza contagiosa, virus-diphtheria, and infectious tracheo-bronchitis. In the presence of a simple nasal discharge and a discharge from the cleft palate, with involvement of the sinuses, and in the absence of typical pustule-like lesions in the mouth and esophagus, there is a close resemblance to coryza contagiosa as well as to a certain stage of virus-diphtheria (Riedmüller (11)). Recent observations have shown that in such cases, from the clinical, gross anatomical, and even etiological aspect, a differentiation is quite impossible. Furthermore in later stages, after the sinuses and the eyes have become involved and when lesions are present in the cavity of the mouth (12), the A-avitaminosis frequently is almost indistinguishable from the commonly occurring roup and virus-diphtheria. The white color of the caseous masses associated with A-avitaminosis is usually considered to be valuable for the differentiation from the more yellowish products in roup and virus-diphtheria; yet these differences, according to our experience, are by no means distinct enough. Finally, in the absence of typical lesions in other organs, difficulties may arise in distinguishing the disease from infectious tracheo-bronchitis which in certain stages shows a very similar picture in the larynx, trachea, and bronchi.

It will be the object of further investigations to discover possible relations between A-avitaminosis and the development of these infectious diseases of the upper respiratory tract. In this problem, as well as for the purposes of differential diagnosis, the most important matter

is a positive recognition of a present lack of vitamin A. The histological lesions which occur in the respiratory tract and throughout the body even in early conditions, taken in connection with the more recent investigations of Wolbach and Howe (5), Goldblatt and Benischek (3), and Tyson and Smith (4), who were able to produce similar lesions in rats and guinea pigs by feeding a diet lacking vitamin A, seem definitely to prove the specificity of these lesions. It may be added that in cases of so-called "coryza contagiosa" without a lack of vitamin A quite different lesions have been found.

Only by means of the histological examination is it possible to differentiate the A-avitaminosis from the above mentioned infectious diseases of the respiratory tract.

SUMMARY AND CONCLUSIONS

1. The principal tissue changes in the respiratory tract of chickens caused by a vitamin A deficiency in the food are, first, an atrophy and degeneration of the lining mucous membrane epithelium as well as of the epithelium of the mucous membrane glands. This process is followed or accompanied by a replacement or substitution of the degenerating original epithelium of these parts by a squamous stratified keratinizing epithelium. This newly formed epithelium develops from the primitive columnar epithelium and divides and grows very rapidly. The process appears to be one of substitution rather than a metaplasia, and resembles the normal keratinization of the skin or even more closely the incomplete keratinization of the mucous membranes (*e.g.*, the esophagus or certain parts of the tongue of chickens). In this connection findings have been described which not only afford an interesting insight into the complicated mechanism of keratinization, but also show probable relations between keratinization and the development of Guarnieri's inclusion bodies. Balloon and reticular degeneration of the upper layers of the new stratified epithelium has been frequently observed. All parts of the respiratory tract are about equally involved in the process; and the olfactory region as well, so that the sense of smell may be lost. The lesions, which first take place on the surface epithelium and then in the glands, show only minor differences.

2. The protective mechanism inherent in the mucous membranes of

the entire respiratory tract is seriously damaged or even entirely destroyed by the degeneration of the ciliated cells at the surface and the lack of secretion with bactericidal properties. Secondary infections are frequently found, and nasal discharge and various kinds of inflammatory processes are common, including purulent ones, especially in the upper respiratory tract, communicating sinuses, eyes and trachea. The development of the characteristic histological process is not dependent upon the presence of these infections, since it also takes place in the absence of infection.

3. The specific histological lesions make it possible to differentiate between A-avitaminosis and some infectious diseases of the respiratory tract.

These studies we hope will serve as a basis for further investigations on the relationship between A-avitaminosis and infection in general.

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EXPLANATION OF PLATES

PLATE 21

FIG. 1. Sagittal section through the head of Chicken 7 with A-avitaminosis. Masses of purulent exudate in the nasal passages. (Dead after 82 days on experimental diet.)

FIG. 2. Cross section through the head of Chicken 13 with A-avitaminosis (spontaneous case). White caseous masses between left concha inferior and nasal septum and in the corresponding sinuses.

FIG. 3. Trachea of Chicken 6 with A-avitaminosis. Thin membranes, partly in the form of a tube, consisting of desquamated epithelium. (Dead after 87 days on experimental diet.)

FIG. 4. Trachea, cross section (84.5 \times). Chicken 1. Beginning stage. Degeneration of the surface epithelium and separation from its underlying support. On the left side formation of a new stratified epithelium in a small focus. (Killed after 30 days on experimental diet.)

FIG. 5. Trachea, cross section (50 \times). Chicken 8. Complete replacement of the epithelium of the respiratory mucosa and its glands by a stratified keratinizing epithelium. Infiltration of the submucosa. (Dead after 80 days on experimental diet.)

PLATE 22

FIG. 6. Same as Fig. 5 (80 \times).

FIG. 7. Cross section through trachea (990 \times). Chicken 1. Newly formed stratified epithelium. At the surface several cells showing balloon degeneration. Foci of bacteria between the degenerating cells. (Dead after 30 days on experimental diet.)

FIG. 8. Nasal septum (50 \times). Chicken 6. Complete replacement of the surface epithelium by stratified keratinized epithelium. Desquamation of the superficial layers. The glands show an earlier stage of the process, atrophy of the original epithelium. Slight infiltration of the submucosa. (Dead after 87 days on experimental diet.)

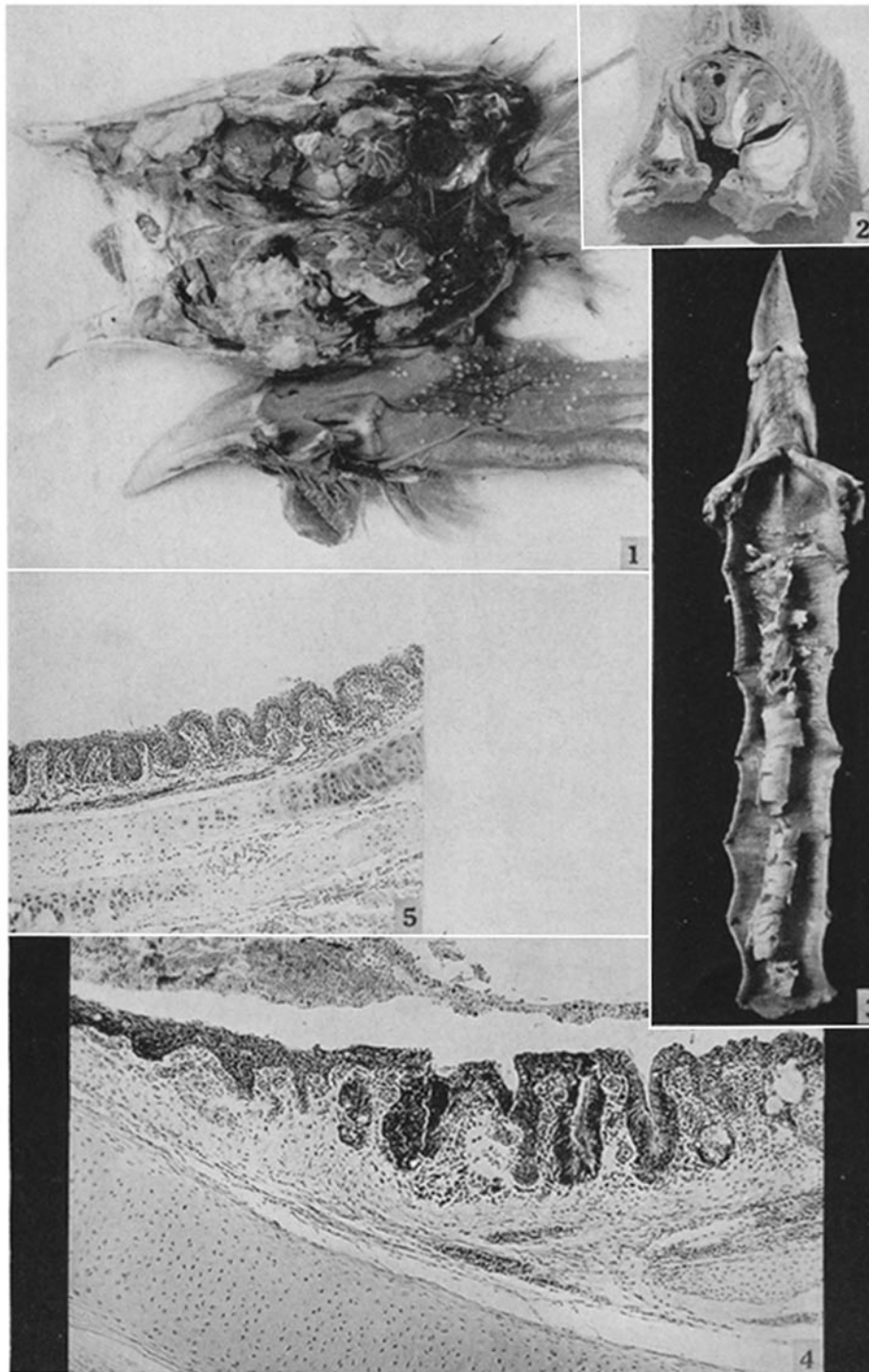
FIG. 9. Olfactory region (85 \times). Chicken 6. Advanced keratinization and desquamation of the replacing epithelium at the surface of the mucous membrane. The epithelium of Bowman's glands shows only the typical atrophy. Masses of exudate and desquamated epithelium. (Dead after 87 days on experimental diet.)

PLATE 23

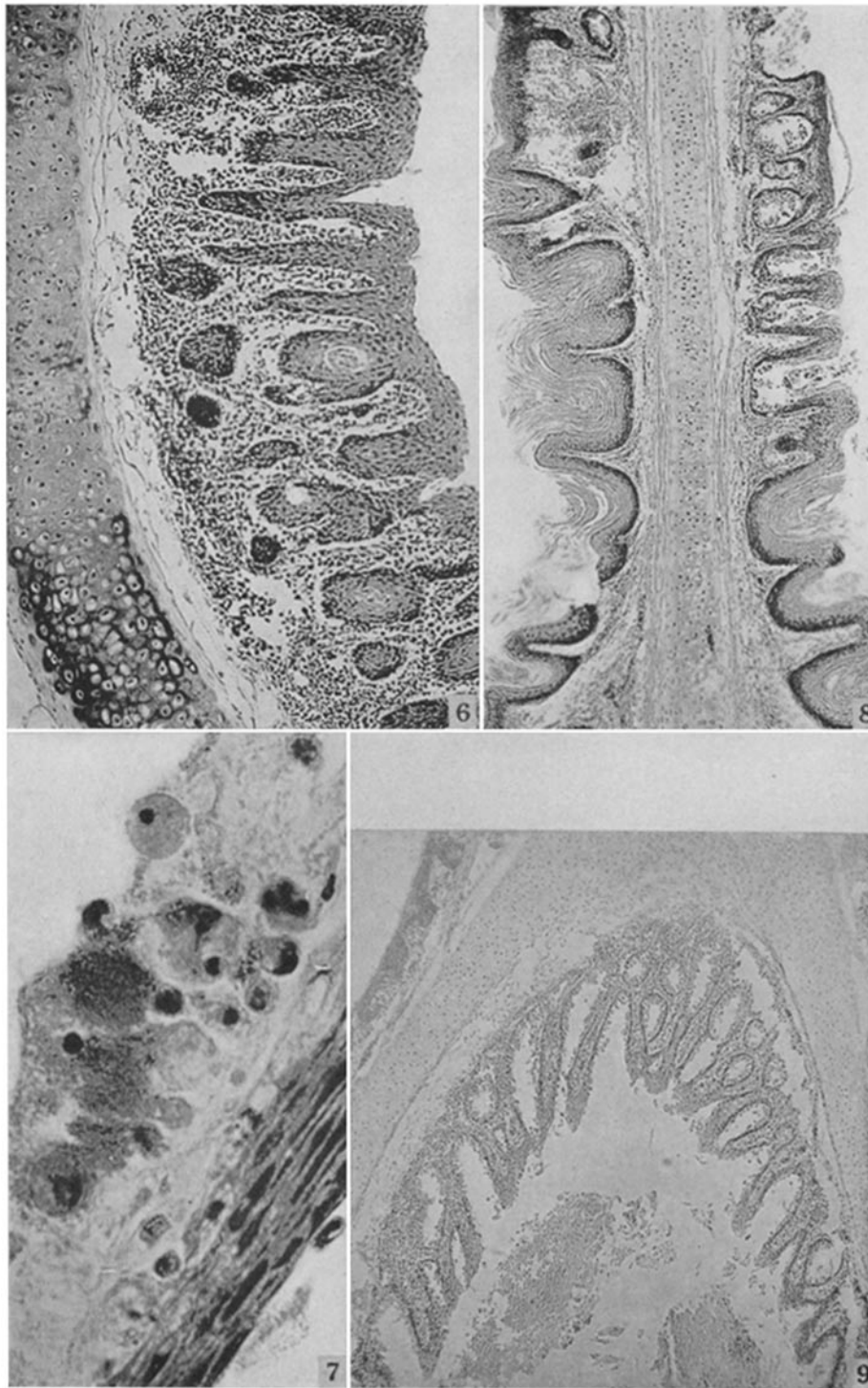
FIG. 10. Respiratory and olfactory mucous membrane of the nasal fossa (55 \times). Chicken 5. Complete replacement by stratified keratinizing epithelium. The glands show an earlier stage of the process. (Dead after 85 days on experimental diet.)

FIG. 11. Gland of the nasal mucous membrane (1140 \times). Chicken 5. Showing numerous bacteria in an early stage of the process. (Dead after 85 days on experimental diet.)

FIG. 12. Nasal passages. Chicken 6. Cells of the superficial layers of the newly formed keratinizing epithelium, showing the extrusion of nuclear material into the cytoplasm (a), granules of keratohyalin (b), masses of eleidin (c), and intercellular bridges (d).



(Seifried: A-avitaminosis in chickens. I)



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